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Authors

Wunk, DF
Werblin, FS

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Synaptic Inputs to the Ganglion Cells in the Tiger Salamander Retina

D. F. WUNK and F. S. WERBLIN

From the Department of Electrical Engineering and Computer Sciences and the Electronics Research Laboratory, University of California, Berkeley, California 94720. Dr. Wunk's present address is Department of Anatomy, Vanderbilt University, Nashville, Tennessee 37232.

ABSTRACT The postsynaptic potentials (PSPs) that form the ganglion cell light response were isolated by polarizing the cell membrane with extrinsic currents while stimulating at either the center or surround of the cell's receptive field. The time-course and receptive field properties of the PSPs were correlated with those of the bipolar and amacrine cells. The tiger salamander retina contains four main types of ganglion cell: "on" center, "off" center, "on-off", and a "hybrid" cell that responds transiently to center, but sustainedly, to surround illumination. The results lead to these inferences. The on-ganglion cell receives excitatory synaptic input from the on bipolars and that synapse is "silent" in the dark. The off-ganglion cell receives excitatory synaptic input from the off bipolars with this synapse tonically active in the dark. The on-off and hybrid ganglion cells receive a transient excitatory input with narrow receptive field, not simply correlated with the activity of any presynaptic cell. All cell types receive a broad field transient inhibitory input, which apparently originates in the transient amacrine cells. Thus, most, but not all, ganglion cell responses can be explained in terms of synaptic inputs from bipolar and amacrine cells, integrated at the ganglion cell membrane.

INTRODUCTION

The aim of this study was to identify and characterize components of synaptic input to each type of retinal ganglion cell in tiger salamander retina. Anatomical studies show that ganglion cells in this animal, like all others studied, receive predominantly chemical synaptic input from both bipolar and amacrine cells (Wong-Riley, 1974). Under normal conditions the light response results from the interaction of excitatory and inhibitory synaptic inputs from the bipolar and amacrine cells. We attempted to isolate individual excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) by separately illuminating antagonistic zones of the receptive field, and polarizing the membrane, thereby augmenting some, while suppressing other PSPs.

Recent studies have suggested that the sustained responses in ganglion cells arise from direct input from bipolars of like phase. Naka (1976) was able to elicit depolarizations in on- or off-ganglion cells of the catfish by depolarizing on- or off-bipolar cells, respectively, with extrinsic currents. Miller and Dacheux (1976 *a-c*) showed that when the retina of the mudpuppy was bathed in low-chloride solution, the responses from the on-bipolar and on-ganglion cells disappeared,

whereas the off-cells continued to respond. Famiglietti et al. (1977) support the notion of separate on and off pathways showing that the terminals and dendritic trees for the on-bipolar and ganglion cells lie in the inner strata, whereas the processes for the off cells lie in the outer strata of the inner plexiform layer in carp. A similar segregation of on and off pathways within strata of the inner plexiform layer has been shown in cat by Nelson et al. (1978).

A role for amacrine cell inhibitory input to ganglion cells has also been proposed. Kaneko (1973) has suggested, based upon spectral sensitivity and receptive field studies that the sustained amacrine cell system in carp forms an antagonistic surround for some ganglion cells. Werblin (1972) has shown that when the change-sensitive amacrine cell system in mudpuppy is depolarized by moving stimuli, the on-off ganglion cells are hyperpolarized. Furthermore, the hyperpolarization is mediated by a conductance increase to ions with an equilibrium potential more negative than the dark potential level (Werblin, 1977). However, the source of the depolarizing transient activity in ganglion cells remains unknown.

This study supports the suggestion that the on-ganglion cells are driven by the on bipolars. Furthermore, it suggests that the on bipolar-to-ganglion cell synapse is excitatory, but *silent* in the dark. Off bipolars appear to drive the off-ganglion cells, also through an excitatory synapse, but which is tonically *active* in the dark. All ganglion cell types are inhibited by a transient IPSP with broad receptive field, probably representing the activity of the amacrine cells. The on-off ganglion cells are excited by an EPSP with narrow receptive field of uncertain origin. Our studies also revealed a fourth kind of ganglion cell, termed "hybrid" here. This cell responds transiently to center illumination, but tonically to surround illumination. It is similar to the ganglion cell response first reported by Naka and Nye (1970) in the catfish. The origin of the synaptic inputs to this cell type is also uncertain at present.

METHODS

The methods used in these experiments are similar to those reported previously (Werblin, 1975; Marshall and Werblin, 1978; Werblin, 1977, 1978). They are reviewed briefly below.

Preparation

Larval tiger salamanders, *Ambystoma tigrinum*, 15–35 cm long, were used in this study. Experimental animals were decapitated, pithed, and enucleated. The anterior portions of the eye, including the ciliary body, were then dissected away, and the vitreous drained with filter paper. The eyecup was placed in a small chamber inside a light-tight shielded cage, and moist, 100% oxygen was passed over the preparation. Experiments were performed at ambient room temperature, about 21°C.

Recording

Cell recordings were made with single- and double-barrel intracellular micropipettes, filled with 3 M potassium acetate (Nelson, 1973; Werblin, 1977; Marshall and Werblin, 1978). Resistances were 350–700 M Ω /barrel. Recording electrodes were connected to a direct-coupled, high-input impedance, capacitance-compensated amplifier, and a chlorided silver wire under the eyecup served as a reference lead. Cell membrane potentials

were measured with respect to the vitral potential. Amplified signals were recorded on a magnetic tape deck (Akai 1730D, Akai America Ltd., Compton, Calif.) with FM recording adapters (Vetter 2D, A. R. Vetter Co., Rebersberg, Penn.).

Light Stimulation

A two-channel photostimulator, using a quartz-iodine lamp (GE 6.6 AT3/4 CL; General Electric Co., Cleveland, Ohio) as source, was used to project light patterns onto the retina; one channel provided a spot, and the other an annulus, of variable dimensions. Maximum irradiance at the plane of the retina was about $40 \mu\text{W}/\text{cm}^2$, between the wavelengths of 4,000 and 7,000 Å. Stimulus intensities were controlled by placing neutral-density filters (Kodak Wrattern 96, Eastman Kodak, Rochester, N.Y.) in the light beams. In this report, all stimulus intensities are specified in log units (LU) with respect to the maximum.

Experimental Protocol

Experiments were performed with the room lights off, and stray background illumination under these conditions was about -6.5 LU. Recording electrodes were advanced from the vitreal side of the retina using a hydraulic microdrive, and the retina was "jolted" (Werblin, 1975) to facilitate penetration of cells by the electrode. While cells were being searched for, the retina was illuminated repeatedly every 8 s with a center flash ($500\text{-}\mu\text{m}$ spot), followed shortly by an annular flash ($700 \mu\text{m}$ i.d./ $2,000 \mu\text{m}$ o.d.), both at -3 LU intensity and 1-s duration. This protocol kept the retina fairly dark-adapted, so the rod and ganglion cell response thresholds were -5 to -6 LU. The stimulus patterns were initially centered on the tip of the recording electrode, but once a cell was penetrated, the stimuli were carefully recentered on the most sensitive area of the cell's receptive field.

Cell Identification

Cells were identified according to their intracellular light responses, and by depth of recording, as established by previous studies, in the retina of the mudpuppy and other species (Kaneko, 1970; Naka and Ohtsuka, 1975; Schwartz, 1974; Werblin and Dowling, 1969; Werblin, 1977). Specific identification criteria are discussed in Results.

The cells referred to herein as transient "amacrine" cells have the response properties of the transient amacrine cells reported in other studies (Werblin and Dowling, 1969; Murakami and Shimoda, 1977), but we have not stained these cells so our identification is tentative. Certain ganglion cells are also known to have similar response properties (Marchiafava, 1976).

Cell Membrane Electrical Measurements

For the measurement of membrane electrical properties, current from a constant current source (Colburn and Schwartz, 1972) was passed through one barrel of an intracellular double-barrel micropipette, while the other barrel monitored membrane potential shifts. The slope of the resulting plot of membrane voltage vs. injected current (V - I) gave a measure of the input resistance of the cell. Because the coupling resistance between electrode barrels appears in series with the membrane resistance (Coombs et al., 1955), it was first subtracted from the measurements before the data were plotted. Coupling resistances of electrodes used were $1\text{--}5 \text{ M}\Omega$, when measured in the extracellular space; the values are apparently not significantly different when the electrodes are inside the cells (Nelson, 1973). All voltage-current curves in this report are steady-state characteristics, as potentials were measured 100 ms or more after the membrane potential stabilized at each current level.

To measure the light response reversal potential and associated resistance change in a cell, light flashes were presented while the membrane was polarized to various potential levels between ± 100 mV. Voltage-current curves were approximately linear in many cells, but were strongly rectifying in some cases. Therefore, to preclude the effects of potential-dependent membrane resistance on light response measurements in those cells (Nelson and Frank, 1967), only those data points in the linear part of the voltage-current curves were used to determine reversal potentials and resistance changes of the light responses.

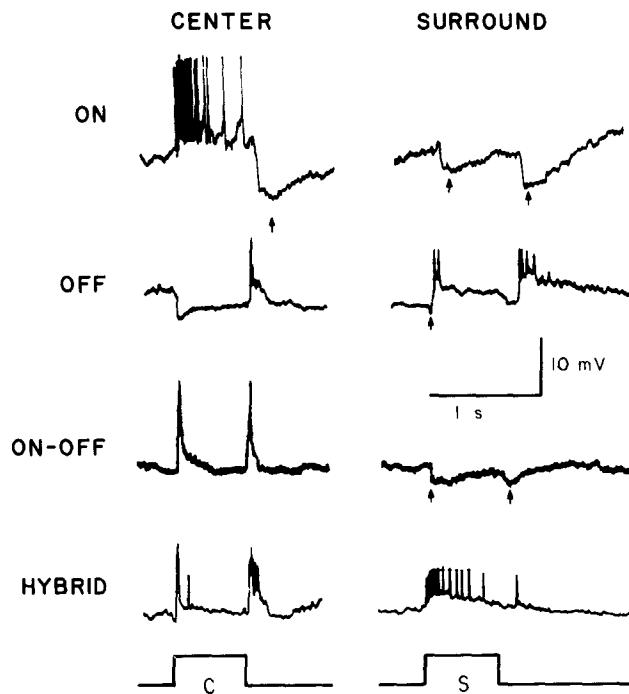


FIGURE 1. Typical response waveforms for the four types of ganglion cell in the retina of the tiger salamander. Responses are characterized in terms of sustained or transient activity to center or surround illumination, as described in the text. Center test disk, $500\text{-}\mu\text{m}$ Diam. Surround annulus, $700 \times 2,000\text{ }\mu\text{m}$. Intensity, -3 log units. Some of the transient hyperpolarizing responses are indicated by the arrows beneath the traces in this and subsequent figures.

RESULTS

Response Waveforms and Stimulus Dimensions

TYPES OF GANGLION CELL RESPONSE The four main forms of ganglion cell response found in the tiger salamander are shown in Fig. 1. They are characterized here on the basis of the transient or sustained response to center or surround illumination at the dark potential level. These responses are enhanced, and other components are revealed, when the membrane is polarized with extrinsic current.

The on-center cells respond with a sustained depolarization to illumination at the receptive field center, but show virtually no sustained response to illumination at the receptive field surround. Most on cells also show transient hyperpolarizations at the onset and termination of the surround stimulus as in Fig. 1.

Off-center cells respond with a sustained hyperpolarization to illumination of the receptive field center, and with a sustained depolarization to illumination at the receptive field surround. There are, in addition, some transient phases to the response at the onset and termination of the stimuli as discussed below.

The on-off cells respond primarily with transient depolarizations to the onset and termination of center illumination, and with transient *hyperpolarizations* to surround illumination. Finally, the "hybrid" ganglion cells respond with transient on-off depolarizations to center illumination, but with a sustained depolarization to illumination of the receptive field surround.

The strategy used in these experiments was to decompose these complex responses into more elementary components and to correlate these components with response waveforms of the cells known to make synaptic contact with the ganglion cells, the bipolar and amacrine cells (Wong-Riley, 1974). Components were identified by illuminating center or surround of the receptive field and then polarizing the membrane with extrinsic current to isolate and accentuate PSPs. Under these conditions the PSPs measured in the ganglion cells had response waveforms and receptive field properties that could be correlated with those of the cell types presynaptic to the ganglion cells, in most cases.

CENTER AND SURROUND RESPONSES OF BIPOLAR AND AMACRINE CELLS
Response waveforms for bipolar and amacrine cells, to center and surround illumination, as found in the retina of the tiger salamander, are shown in Fig. 2. The on-bipolar cell is depolarized by illumination at its receptive field center, and hyperpolarized by illumination at its receptive field surround. Similarly, the off-center bipolar cell is hyperpolarized by illumination at the receptive field center and depolarized by illumination at its receptive field surround. This result is slightly different from that reported in the mudpuppy (Werblin and Dowling, 1969; Werblin, 1974), where bipolar cells show a surround response *only* when the receptive-field center is also being illuminated. Bipolars in frog (Matsumoto and Naka, 1972), fish (Kaneko, 1973), and turtle (Yazulla, 1976; Richter and Simon, 1975; Schwartz, 1974) show the separate surround response, similar to that seen in tiger salamander.

The variety of amacrine cell most commonly recorded in the retina of the tiger salamander is the transient type. It usually has a broad receptive field, and responds transiently, at on and off, to both center and surround stimuli (Fig. 2). The sustained form of amacrine cell response, as found in fish (Kaneko, 1973; Naka and Ohtsuka, 1975; Murakami and Shimoda, 1977) and frog (Matsumoto and Naka, 1972), has recently been reported in salamander retina (Chan and Naka, 1976; Vallerga¹) but was recorded only very rarely in these studies. Sustained amacrine cells do not have concentric antagonistic receptive fields, and can therefore be easily separated from bipolar cells.

¹ Vallerga, S. Personal communication.

The three response types shown in Fig. 2, arising from identifiable cell types which are precursors to ganglion cell activity, can be correlated with most of the PSP waveforms measured in ganglion cells presented below, with one exception. Some ganglion cells appear to receive a transient depolarizing input with *narrow* receptive field, similar to the amacrine cell response, but with no response to the test annulus (shown schematically in Fig. 2, lowest trace). This is not attributable to a single cell type found in our experiments, and its origin is presently unknown. It may represent, for example, the interaction between bipolars or amacrine cells presynaptic to the ganglion cell membrane.

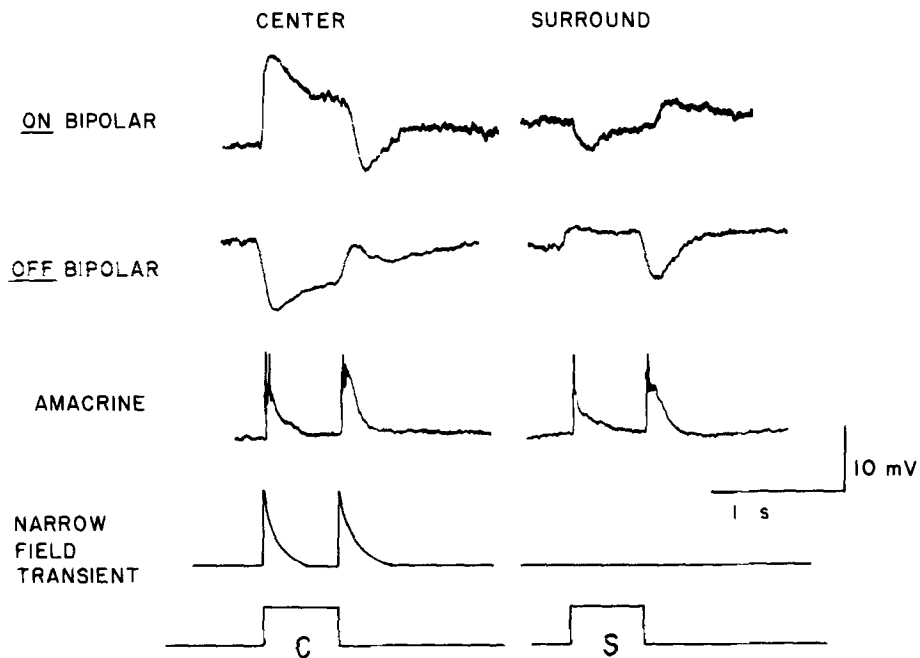


FIGURE 2. Typical center and surround response waveforms for bipolar and amacrine cells. Center test disk, 500 μm ; surround annulus, 700 \times 2,000 μm . Intensity, -3 log units. In addition, a narrow-field transient input, inferred from ganglion cell recordings, is shown in the lowest row. This does not correspond to a recorded presynaptic cell type, but may represent interacting inputs of cells presynaptic to the ganglion cell.

In the following sections, PSPs resembling the waveforms shown in Fig. 2 were measured during polarization of the ganglion cell membranes. The similarities in waveform were used to infer the cellular origin of the synaptic inputs. First we present data to justify the use of center test stimulus with 500- μm Diam and surround annulus of 700 μm i.d., used to separate center and surround components of the ganglion cell response.

MEASUREMENT OF RECEPTIVE FIELD CENTERS FOR THE BIPOLAR AND AMACRINE CELLS The transient components of the responses of bipolar and amacrine cells occur during the first 200 ms of the response, as shown in Fig. 2.

When synaptic inputs from both cell types impinge upon a ganglion cell, there will be some overlap of the effects of each, making the identification of the inputs difficult. However, it was possible to choose a dimension for the center and surround stimuli that assured that the bipolar response was relatively "square," so that the PSP measured during the sustained phase of the ganglion cell response could be assigned to the bipolar input.

Stimulus intensity also affected the form of the response. Dim stimuli elicited slow, poorly defined responses (Wunk, 1977), whereas bright stimuli could not be confined to any region of the receptive field because of light scatter. Therefore, test stimuli at both center and surround, of intensity that elicited a near-maximal center response, were used throughout this study.

Response waveforms generated at different test flash diameters are shown in Fig. 3 for the two types of bipolar and the amacrine cell. The bipolar responses

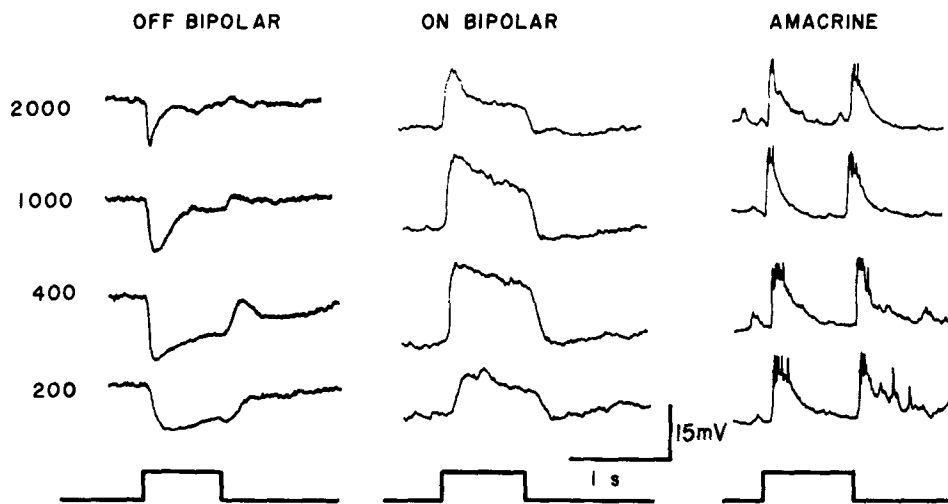


FIGURE 3. Responses of bipolar and amacrine cells to test disks of different diameter. The bipolar responses are sustained to test disks of 400 μm and less. Larger test disks elicit a peak-plateau sequence. Amacrine cell responses are of similar magnitude for all stimulus dimensions.

were "square" and increased in magnitude for stimulus diameters up to 400 μm . For larger diameter test stimuli, the bipolar responses became more transient and tended to lose the sustained phase. Conversely, the amacrine cell responses were transient for all test stimulus diameters.

MEASUREMENT OF RECEPTIVE FIELD CENTERS FOR THE GANGLION CELLS The 400- μm stimulus diam, chosen for eliciting simple sustained responses in the bipolars, was also appropriate for eliciting sustained responses in some of the ganglion cell types. Fig. 4 shows the responses recorded in the four types of ganglion cell to test flashes of different diameters. As with the bipolar cells, the sustained component of responses of the on-center and off-center ganglion cells increased in magnitude for increasing test flash diameters up to about 400 μm . For larger test flash diameters the responses became more complex, showing

peak and plateau after stimulus onset, and, in most cases, hyperpolarizing transients at the onset and termination of the stimulus.

The response of the on-off ganglion cell was dominated by an initial depolarizing transient followed by hyperpolarizations for small test spot diameters at on and off. For large spot diameters, the transient depolarization was lost and the hyperpolarizing response predominated. The depolarizing response was largest for test spot diameters near 400 μm .

Hybrid ganglion cells showed the same increase in complexity as the stimulus diameter was increased beyond 400 μm . The response changed from depolarizing transients at on and off to a biphasic series of transients for larger test spot diameters.

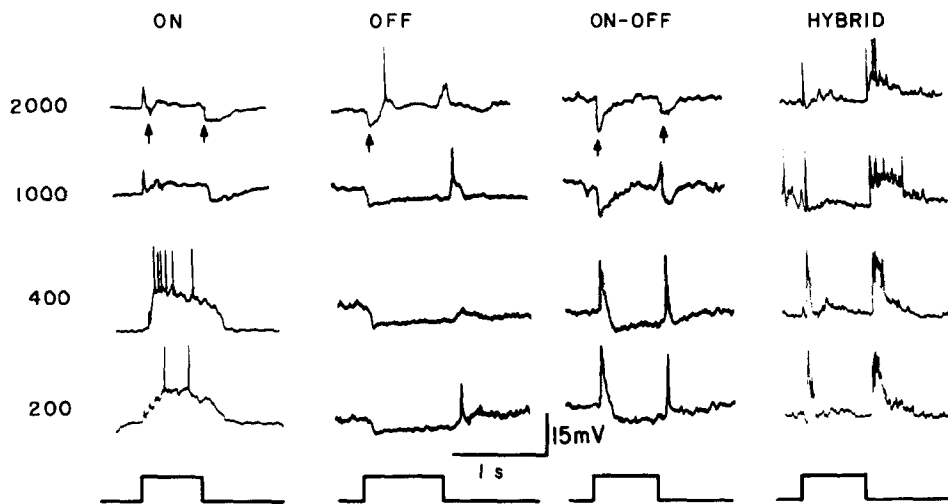


FIGURE 4. Responses of the four ganglion cell types to test disks of different diameter. On-center and off-center cells respond with relatively sustained polarizations to test disks below 400 μm Diam. Transient components appear for larger test disks. On-off ganglion cell response is converted from transient depolarizations to transient hyperpolarizations as the test disk increases in diameter beyond 400 μm . The arrows mark the more apparent transient hyperpolarizations.

In summary, results taken from many bipolar, amacrine, and ganglion cells show that test center spots of 400- to 600- μm Diam at intensities that elicit near-maximal responses in all cell types lead to relatively pure sustained polarizations in the bipolar cells and in the on-center and off-center ganglion cells. Much larger stimulus diameters or greater stimulus intensities elicited complex response waveforms in the ganglion cells, less similar to those recorded in the bipolars. We therefore selected a receptive field center stimulus diameter of 500 μm for all experiments, anticipating that this stimulus would elicit sustained responses along the bipolar-to-ganglion cell pathway, leaving the responses relatively free from lateral interactions due to input from surrounding retinal regions. The surround pathways were driven by a test annulus of 700 μm i.d.

and 2,000 μm o.d. designed to avoid the receptive field center, but to illuminate most of the surround.

Effects of Membrane Polarization on the Ganglion Cell Responses

COMPONENTS OF THE ON-CENTER GANGLION CELL RESPONSE The on-center ganglion cell response appears to consist of two components: a tonic depolarization elicited only by center illumination, and a transient hyperpolarization at on and off elicited by both center and surround illumination. The transient

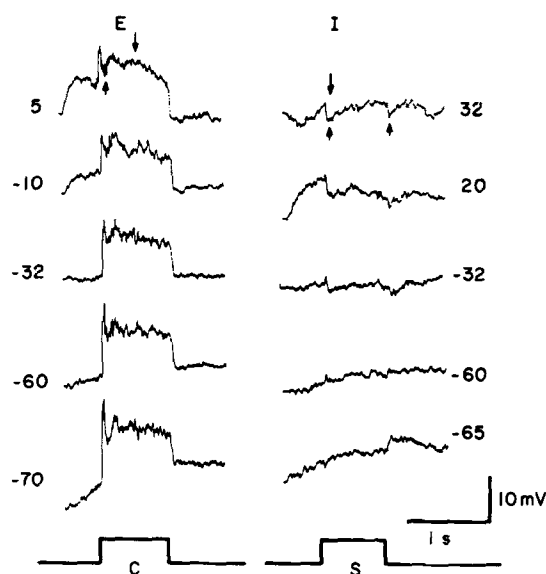


FIGURE 5. Responses of on-center ganglion cell to center and surround illumination at different potential levels. Center response consists of a sustained depolarization decreasing with steady membrane depolarization. Surround response consists of transient hyperpolarizations which reverse near -60 mV. Numbers to the side of recordings show membrane potential in dark. No current potential level was -32 mV. Center test disk diameter, $500 \mu\text{m}$; surround test annulus, $700 \times 2000 \mu\text{m}$; stimulus intensity, -3 log units in this and subsequent figures. Arrows associated with *E* and *I* show times where components of the response were measured at each potential. Arrows beneath the traces indicate transient hyperpolarizations.

hyperpolarizations are not apparent in all records of the center response, but a survey of many recordings suggests that they are frequently present. In Fig. 5 and subsequent records, these hyperpolarizations are identified by arrows beneath the traces. The following response vs. membrane potential data indicate that the tonic depolarization and the transient hyperpolarizations are EPSPs and IPSPs mediated by conductance increases at the ganglion cell membrane.

Responses to center and surround illumination at several membrane potentials are illustrated in Fig. 5. The spike activity in this cell, like many others, disappeared shortly after penetration, but the slow potential changes associated

with the response persisted throughout the experiment. Center illumination elicited primarily a sustained depolarization that decreased when the membrane was depolarized and increased with membrane hyperpolarization. Surround illumination elicited no sustained response, but hyperpolarizing transients, indicated by arrow beneath the traces, could be measured consistently, and increased in magnitude with membrane depolarizations. Some drift was noticeable in the membrane potential records, attributed here to changes in the current-passing characteristics of the electrode.

We choose to measure the magnitude of sustained depolarization at the time marked *E* in Fig. 5 because the presumed concurrent transient hyperpolarizing influence has decayed at this time. Some component of the transient hyperpo-

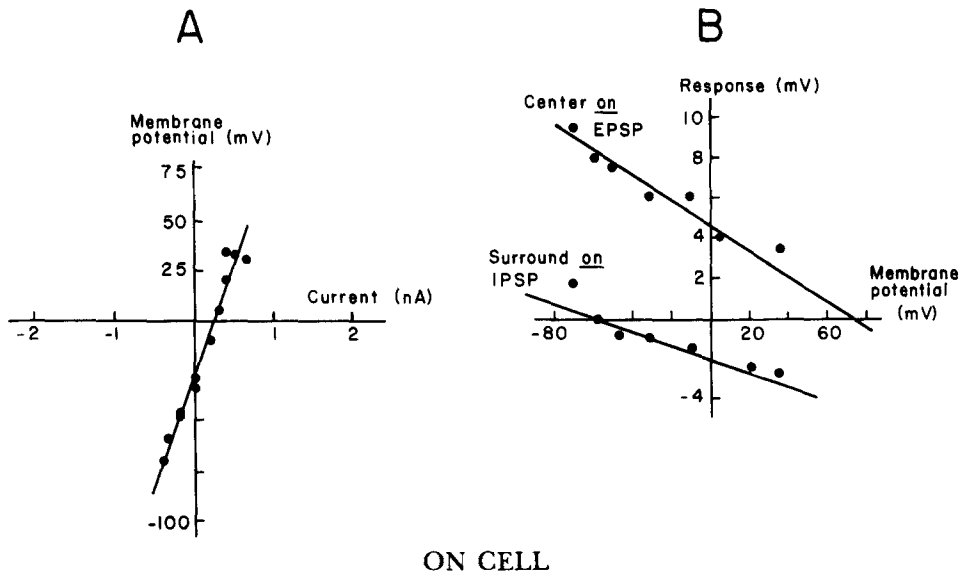


FIGURE 6. (A) Voltage-current relation for on-center ganglion cell membrane. Slope resistance near $108 \times 10^6 \Omega$. (B) Response vs. potential for times *E* and *I* shown in Fig. 5. Points taken from time *E* indicate an EPSP with reversal potential near +75 mV; points from *I* show an IPSP with reversal potential near -60 mV.

larization may still exist, and will distort the measurement of the magnitude of depolarization as a function of membrane potential. The magnitude of the hyperpolarizing response was measured at the time marked *I*. There is no evidence for a concurrent sustained response at this time.

The voltage-current curve for this cell, shown in Fig. 6 A, indicates that the membrane had a linear slope resistance near $108 \times 10^6 \Omega$ and a dark potential of -32 mV. The response vs. potential curves for the PSPs are shown in Fig. 6 B. The depolarization, elicited by center illumination at the time marked *E* in Fig. 5, appears to be an EPSP due to a resistance decrease of $5.7 \times 10^6 \Omega$ and extrapolated reversal potential near +75 mV. The transient hyperpolarizations, measured at the time marked *I* in Fig. 5 appear to be components of an IPSP

associated with a resistance decrease of $3.1 \times 10^6 \Omega$ and reversal potential near -60 mV. Average reversal potentials and resistance changes associated with the PSPs are shown in Table I for 10 on-center ganglion cells.

The transient hyperpolarizations elicited by the onset of surround illumination also appear at the termination of the surround stimulus, and have a similar reversal potential to the component indicated by *I* in Fig. 5. Although not always apparent in each record, it is our impression that the transient hyperpolarizations also exist in some cells at the onset and termination of the *center* test flash

TABLE I
SUMMARY OF GANGLION CELL MEMBRANE ELECTRICAL MEASUREMENTS

Cell type	Number	Dark potential mV	Input resistance $\Omega \times 10^6$	Reversal potentials		Resistance changes	
				EPSP mV	IPSP mV	EPSP $\Omega \times 10^6$	IPSP $\Omega \times 10^6$
On-center	10/8	-27 ± 8	82 ± 35	30 ± 25	-43 ± 24	-7.1 ± 5.1	-5.1 ± 3.6
Off-center	8/5	-34 ± 6	81 ± 44	52 ± 30	-59 ± 12	-5.3 ± 3.7	-14.4 ± 16.5
On-off center	11/11	-24 ± 9	66 ± 19	28 ± 27	-45 ± 10	-7.3 ± 3.3	-9.4 ± 5.6
Hybrid	7/3	-40 ± 18	53 ± 34	6 ± 22	-47 ± 6	-11.2 ± 4.2	-6.5 ± 3.2

Means \pm SDs.

In the "number" column, the first number represents total cells studied; the second number represents those in which IPSPs were observed.



FIGURE 7. Response of another on-center ganglion cell at two potential levels. At the dark potential level of -25 mV, transient hyperpolarizations at on and off of center and surround for stimuli are apparent. The arrows indicate hyperpolarizations at each response phase. At -55 mV, the transient hyperpolarizations are absent, and the response resembles that of the on-bipolar cell shown in Fig. 2. Measured dark potential level -25 mV may be inaccurate, because cell resumes spiking near -55 mV.

as indicated by the arrow beneath the top trace in Fig. 5. An example of these transients is also given in Fig. 7. When the membrane was held at -55 mV, near the reversal potential for the IPSPs as determined in Fig. 6, there is no sign of any transient hyperpolarization in either the center or the surround response. At -25 mV, however, the transient hyperpolarizations are evident in the response to surround illumination. We also believe that this configuration of transient hyperpolarizations exists in the center response at -25 mV. It obscures much of the sustained depolarization and contributes to the transient hyperpolarizing response at the termination of the center stimulus.

The IPSPs are easy to "read" in the surround responses because there is no overlap with a concurrent sustained response component. The presence of the IPSPs in the center response is more difficult to ascertain, so we present it here only as a suggestion. We have marked its possible existence during center illumination with the arrows beneath the traces in Figs. 1, 5, and 7.

The tonic excitation elicited by center illumination is probably due to bipolar input, because bipolars and on-ganglion cells have the same receptive field properties (cf. Figs. 3 and 4), and bipolars are the primary sustained presynaptic cells. The transient inhibition is probably due to input from the amacrine cell system, which appears to have a broad receptive field and characteristically

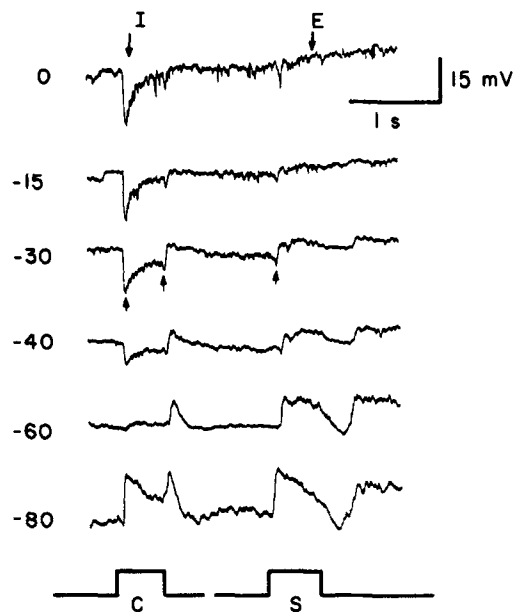


FIGURE 8. Response of off-center ganglion cell. Center response consists of a prominent transient hyperpolarization which reverses polarity near -55 mV. Surround response consists of a steady depolarization which decreases with membrane depolarization and small transient hyperpolarizations (arrowheads) which reverse polarity near -55 mV. Dark potential level, -40 mV.

transient response. These inferences are justified more thoroughly in the Discussion.

COMPONENTS OF THE OFF-CENTER GANGLION CELL RESPONSE The off-center ganglion cell response, illustrated in Figs. 1 and 8, consists of a sustained hyperpolarization to center illumination, and a sustained depolarization to surround illumination. In addition, there is some evidence for transient hyperpolarizations associated with the onset and termination of the center and surround stimuli. The tonic responses appear to result from modulation of an ongoing excitatory input, whereas the transient responses appear to be IPSPs, as inferred from the response vs. membrane potential data below.

Responses of an off-center ganglion cell to center and surround illumination at several membrane potentials are shown in Fig. 8. The sustained depolarizing responses to surround illumination, at the time marked *E* are the easiest to follow. They increase with membrane hyperpolarization and decrease with membrane depolarization, becoming almost unmeasurable near 0 mV. The sustained hyperpolarizations to center illumination are more difficult to interpret because they are obscured by a strong hyperpolarizing transient at the onset of the center stimulus that decays slowly throughout the center response. The magnitude of this transient is also a function of membrane potential.

Our interpretation of the transient center response is consistent with the data from the other cell types, where the hyperpolarizing transients always seem to reverse near -50 mV. The transient at center on is most strongly hyperpolariz-

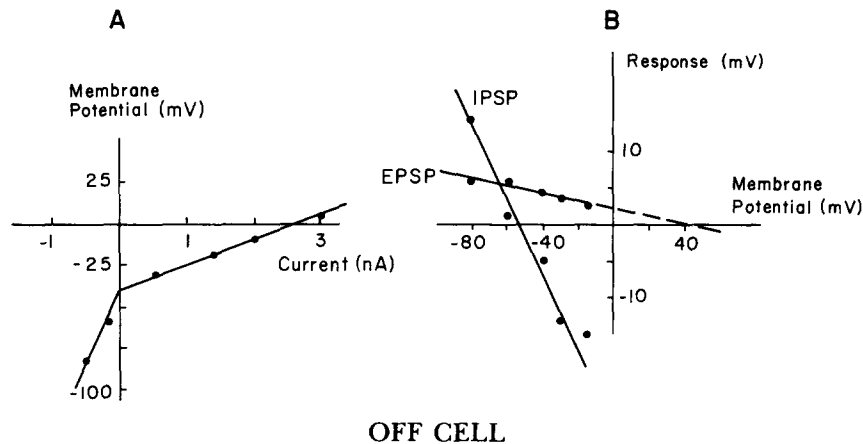


FIGURE 9. (A) Voltage-current curve for off-center ganglion cell shown in Fig. 8. (B) Response vs. potential curves taken from responses in Fig. 8 at times *E* and *I*. Because the membrane is outward rectifying, only data taken at potentials more negative than -15 mV were used. Responses taken at time *E*, during the sustained depolarization, represent an EPSP with extrapolated reversal potential near $+46$ mV. Responses at time *I*, during the hyperpolarizing transients at center on, represent an IPSP with reversal potential near -55 mV.

ing at 0 mV, and most strongly depolarizing at -80 mV. The off-center response at -60 mV is almost absent because the sustained hyperpolarization is obscured by the small presumed depolarizing transient at this level. The responses in Fig. 10 show the sustained hyperpolarizations to center illumination more clearly, because the transients in this cell are relatively small and nearly absent at -55 mV. Even in Fig. 10, the presumed large sustained hyperpolarizing response to the center stimulus at -100 mV is obscured by the concomitantly large depolarizing transient at center on.

The current-voltage curve for the off-center ganglion cell in Fig. 8 is shown in Fig. 9 A. The membrane is strongly outward rectifying, so the data for response magnitude as a function of membrane potential are only useful for potentials more negative than -30 mV. These data suggest that the sustained depolariza-

tion to surround illumination is an EPSP with reversal potential near $+40$ mV, and associated with a decrease in membrane resistance of $4.4 \times 10^6 \Omega$. The IPSP, measured for the response transient at center on in Fig. 8, has a reversal potential near -55 mV and is associated with a resistance decrease of $43.4 \times 10^6 \Omega$.

Figs. 8 and 10 show that the relative magnitudes of the IPSPs at different phases of the response vary from cell to cell. In Fig. 8 the IPSP is large at center on, small at center off and at surround on, and nearly unmeasurable at surround off. In Fig. 10 the IPSP is smaller at center on, small at surround off, and nearly nonexistent at the other phases. A survey of all off-center cells studied suggests that the transient IPSP can exist at any or all of the four possible points in time during the responses, and when it is large enough to be followed at different membrane potentials, the IPSP reverses polarity near -59

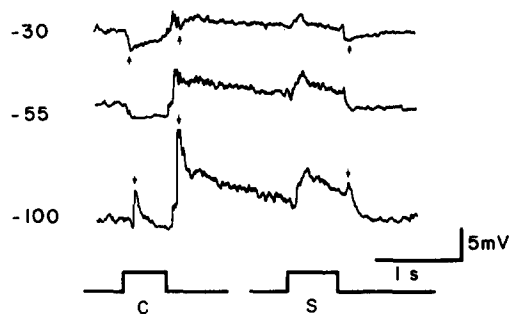


FIGURE 10. Another off-center ganglion cell showing more clearly a sustained hyperpolarization during center illumination and depolarization during surround illumination that does not reverse at the IPSP reversal potential (-55 mV). In this and other cells the extrapolated reversal potentials for sustained hyperpolarizations and depolarizations elicited by center and surround illumination, respectively, averaged 52 – 78 mV, suggesting that the responses may be of common origin. Arrows locate possible IPSPs.

mV. Table I shows the average measurements for eight off-center ganglion cells studied in this way.

RESPONSE COMPONENTS OF THE ON-OFF GANGLION CELLS The response of the on-off ganglion cell appears to consist of at least two components: center illumination elicits a transient depolarization at on and off; both center and surround illumination elicit transient hyperpolarizations at both on and off. The data below suggest that the transient depolarization is due to an EPSP with narrow receptive field, whereas the hyperpolarizations are due to transient IPSPs with broad receptive field.

Responses of a typical on-off ganglion cell to center and surround illumination at six different membrane potential levels are shown in Fig. 11. As in other cells, the spiking response disappeared shortly after penetration, leaving only the slow potential responses. However, in this cell some spikes reappeared when the membrane was hyperpolarized to an apparent level near -75 mV. This suggests that the membrane may have been damaged (and depolarized) by the

penetration, and that the dark potential level may actually be more negative than the -25 mV measured here.

The on-center response appears to consist of a concurrent EPSP and an IPSP, so there is no simple way to extract either component. The response to center illumination at the measured dark potential level near -25 mV appears biphasic, but most of the other responses are simpler. The excitatory component appears to precede the inhibitory component, so an estimate of the excitatory response was taken at its peak, at the time marked *E* in Fig. 11. The surround hyperpolarizing transient response is easier to analyze because there is no concurrent excitation. Measurements of response magnitude were taken at the

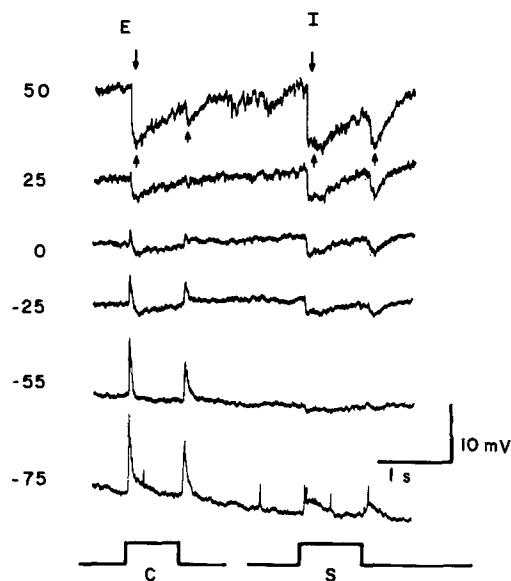


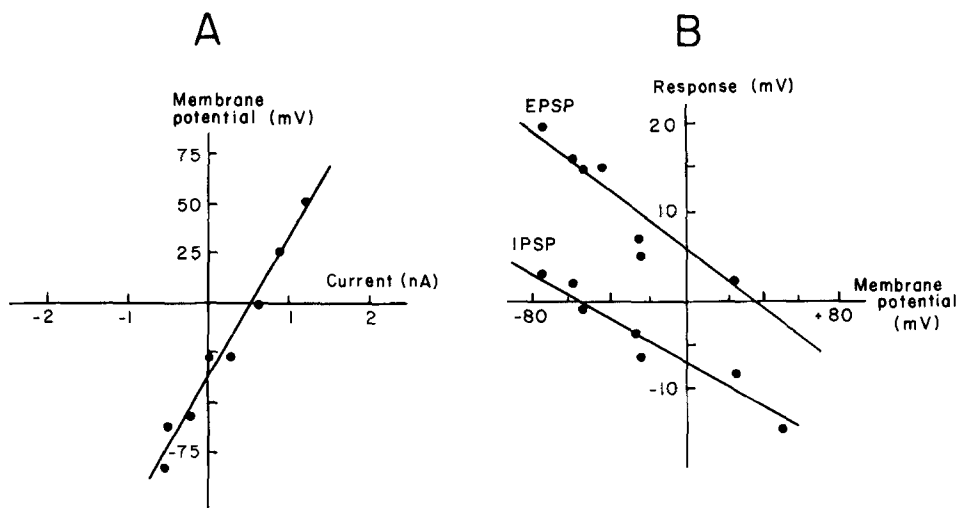
FIGURE 11. Responses of on-off ganglion cell to center and surround illumination at different membrane potential levels. Center response consists of transient depolarizations which become transient hyperpolarizations as the membrane is depolarized. Responses at -25 and 0 mV are biphasic, suggesting multiple sources. Surround response consists of transient hyperpolarizations which reverse polarity near -55 mV. Dark potential near -25 mV. Spiking resumed in this cell near -75 mV. Arrows indicate possible IPSPs.

time marked *I* in the figure. The response magnitudes are plotted against membrane potential in Fig. 12.

The voltage-current curve for the membrane in the dark is shown in Fig. 12 A. The membrane resistance is linear near $70 \times 10^6 \Omega$. The response vs. potential for the PSPs are shown in Fig. 12 B. The EPSP has a reversal potential near $+35$ mV and is associated with a resistance decrease of $11 \times 10^6 \Omega$. The IPSP has a reversal potential of -55 mV and is associated with a resistance decrease of $8 \times 10^6 \Omega$. The measurements for the EPSP are probably underestimates of the true values because of some unavoidable contribution from the concurrent IPSP in the measurement of the on response to center illumination.

In this and other cells the transient IPSPs were always present at the onset and termination of both center and surround responses. The reversal potential for this component always fell near -45 mV. This appears to be a component similar to that found in the on- and off-center ganglion cells, but its presence in all phases is more consistent in the on-off cell. The origin of this IPSP is probably the broad field, transient amacrine cell system.

The transient EPSPs have apparently a narrow receptive field. There is no clear presynaptic candidate for this component of the response. Therefore, the EPSP may represent the presynaptic interaction of bipolar or amacrine cells which form a synaptic input not measurable in any single cell type. Possible sources of the narrow-field transient EPSP are indicated in the Discussion.



ON-OFF CELL

FIGURE 12. (A) Voltage-current curve for the membrane of the on-off cell shown in Fig. 11. It is linear with slope resistance near $70 \times 10^8 \Omega$. (B) Response vs. membrane potential measured at times *E* and *I* in Fig. 11. The transient depolarization at *E* is an EPSP with reversal potential near $+35$ mV. The transient hyperpolarization at *I* is an IPSP with reversal potential near -55 mV.

RESPONSE COMPONENTS OF THE HYBRID GANGLION CELL The hybrid ganglion cell appears to have the most complex synaptic input of the ganglion cells studied in the tiger salamander. At least three separate response components can be identified. As in many other ganglion cells, transient IPSPs can be measured at both onset and termination of both center and surround stimuli. In addition, center illumination elicits a *transient depolarization* at both on and off, similar to that of the on-off center cell. Finally, surround illumination elicits a *sustained depolarization*, similar to that of the off-center cell.

Fig. 13 shows the responses of a hybrid ganglion cell to test flashes at center and surround, while the membrane was polarized to different potential levels. The response at the dark level, near -35 mV, shows the typical transient center

and sustained surround depolarizations. Both of these depolarizations increase in magnitude as the membrane is hyperpolarized. When the membrane was depolarized, the depolarizations decrease in magnitude and transient hyperpolarizations at on and off of center and surround stimuli appear. These transient hyperpolarizations increase with further membrane depolarization.

Three components of the response were identified in these records and measured at each potential level: the transient depolarization at center on, labeled E_t , the sustained depolarization during surround illumination, labeled

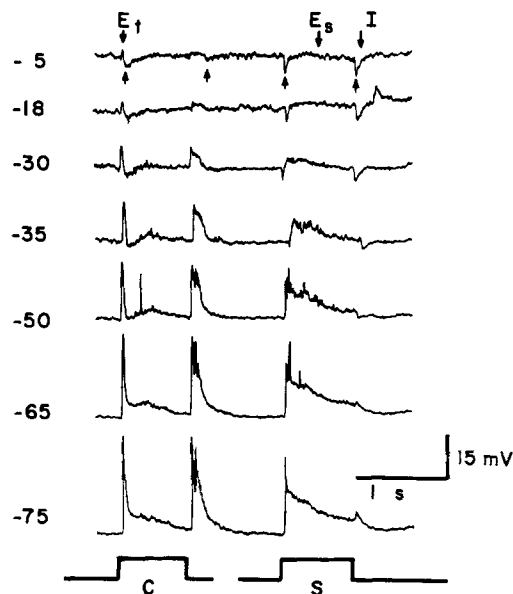


FIGURE 13. Responses of hybrid ganglion cell to center and surround illumination at various membrane potentials. Center response consists of a transient depolarization at on and off which decreases in magnitude as the membrane is depolarized, and is biphasic near 0 mV. Surround response consists of a sustained depolarizing component which decreases in magnitude as the membrane is depolarized. In addition, the arrowheads indicate times for transient hyperpolarizing components which are obscured near the dark potential level of -35 mV, hyperpolarizing near 0 mV, and depolarizing at membrane potentials more negative than the dark potential level.

E_s , and the transient hyperpolarization at the termination of the surround stimulus, labeled I . These measurements are plotted against membrane potential in Fig. 14 B.

Fig. 14 A shows that the membrane resistance for this cell was slightly outward rectifying, with resistance near the dark level at $22 \times 10^6 \Omega$. Fig. 14 B shows that the three response components are all associated with resistance decreases. The transient and sustained depolarizations have reversal potential near 0 mV, whereas the transient IPSP had a reversal potential near -55 mV.

The transient hyperpolarizations measured in this and other hybrid cells could appear at all phases of the response. When they were large enough to follow through different membrane potential levels, they usually reversed at about -47 mV, near the reversal potential for all other transient hyperpolarizations in this report. The EPSPs in the hybrid cell had reversal potentials near 6 mV, somewhat lower than those measured in the other cell types. The slight rectification of the membrane and some interference by the nearly concurrent IPSP could contribute some error to this measurement leading to an underestimation of the true reversal potential for the excitatory responses.

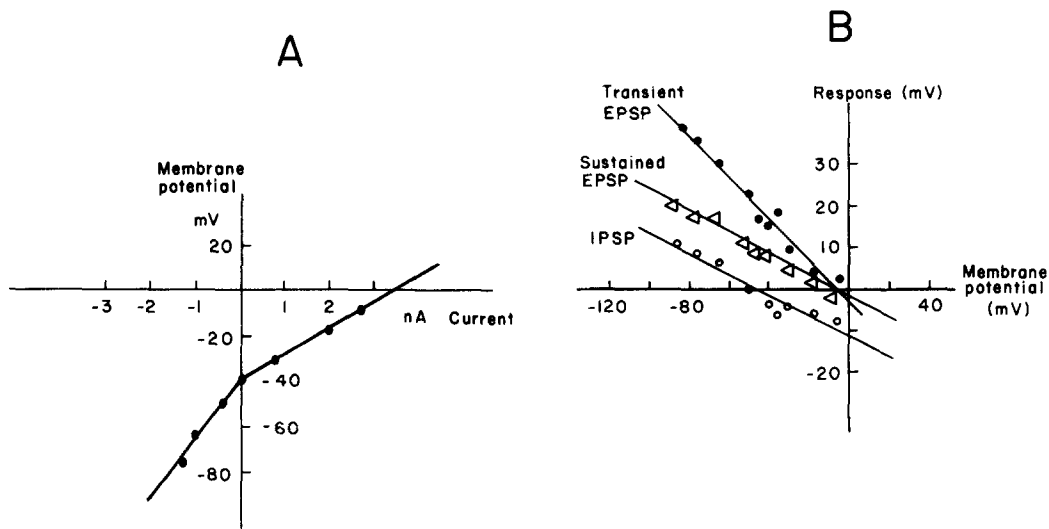


FIGURE 14. (A) Voltage-current curve for hybrid ganglion cell in the dark. (B) Response vs. potential level for three components of the hybrid ganglion cell response shown in Fig. 13. The transient depolarizing component measured at time E_t appears to be an EPSP with reversal potential near 0 mV. The sustained depolarization measured at time E_s appears to be an EPSP with reversal potential near 0 mV. The IPSP, seen in maximal isolation at the termination of the surround stimulus, has a reversal potential near -50 mV.

DISCUSSION

Identification of the Cell Types Making Synaptic Input to the Ganglion Cells

SUSTAINED INPUTS TO THE ON-CENTER AND OFF-CENTER GANGLION CELLS ARE EXCITATORY Three earlier studies suggest that the on bipolars impinge upon the on-ganglion cells, and that the off bipolars drive the off ganglion cells. Naka (1976) showed this in catfish by polarizing bipolars with extrinsic current while recording from the ganglion cells. Miller and Dacheux (1976 *a-c*) showed this in mudpuppy by suppressing activity in the on bipolars with low extracellular chloride and showing the disappearance in mudpuppy of the response in the on- but not the off-, ganglion cells. Finally, Famiglietti et al. (1977) in fish, and Nelson et al. (1978) in cat showed that the on-bipolar terminals and the on-

ganglion cell dendrites lie in a different strata of the inner plexiform layer than the processes of the off-bipolar and ganglion cells. These studies strongly support the notion that there are separate functional and anatomical on and off pathways in the retina that are organized before the level of the inner plexiform layer.

Our study is directed toward a complementary issue: what is the synaptic mechanism along each pathway at the inner plexiform layer, and what is the total complement of synaptic inputs impinging upon each ganglion cell type? The probable sources of the PSPs measured in the ganglion cells, inferred from

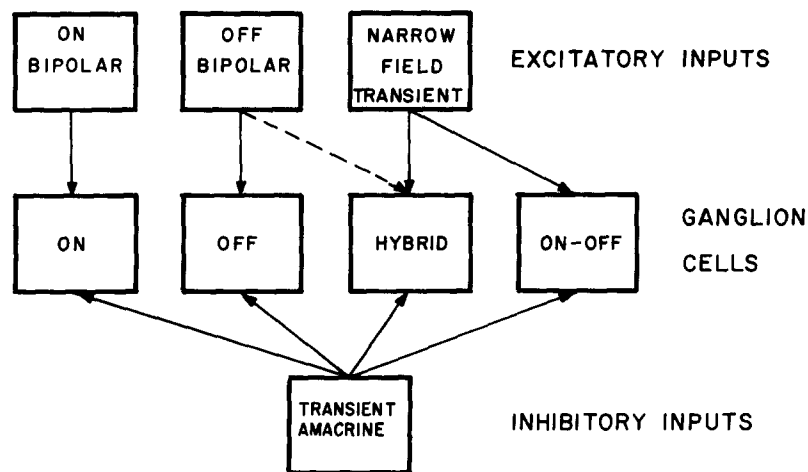


FIGURE 15. Summary diagram for synaptic inputs to the ganglion cells. The presumed cell types providing the excitatory inputs are shown above; inhibitory inputs are shown below. On bipolars excite the on-ganglion cells, off bipolars excite the off-ganglion cells. Narrow field transient excitatory inputs are found in on-off and hybrid cells; the source of these inputs is uncertain. The excitatory sustained input to the hybrid cells (dashed arrow) is also of uncertain origin. All ganglion cells are transiently inhibited by a broad field system presumed to originate in the transient amacrine cells. There is no evidence for bipolar cells making direct inhibitory connections with the ganglion cells. The action of synaptic transmitter at each of the synapses shown is apparently to increase permeability in the respective ionic channel of the ganglion cell membrane.

the data in this report, and correlated with other studies, are summarized in the diagram in Fig. 15. This report suggests that if the hypotheses of the connections outlined above are correct, the synaptic inputs to the on- and off-ganglion cells, derived from their respective bipolars, are both excitatory.

The hybrid ganglion cell also generates a sustained depolarization in response to surround illumination, closely resembling the surround response of the off-center ganglion cell. The hybrid cells, however, show little sign of a sustained hyperpolarization in response to center illumination. Without the correlative evidence from other studies, it is not possible at this time to ascertain the

identity of the source of sustained surround input to the hybrid cells. The dashed line in Fig. 15 represents our tentative suggestion of input from the off-center bipolar cell.

ON PATHWAY IS SILENT; OFF PATHWAY IS ACTIVE IN THE DARK The surround responses of the on-center ganglion cell shown in Figs. 1, 5, and 7 show little sign of a sustained hyperpolarization, although the surround responses of the on-bipolar cells (Fig. 2) do show a sustained hyperpolarization. The loss of the hyperpolarizing response to surround illumination at the ganglion cell might occur because the synapse from bipolar to on-ganglion cell is *silent* in the dark. Conversely, in the off-center ganglion cell, a clear sustained hyperpolarizing response is measured to center illumination and a sustained depolarizing response is measured to surround illumination (Figs. 1 and 8). Inasmuch as this synapse also appears to be excitatory (Fig. 9), the sustained responses are probably mediated by *modulation* of an *ongoing* synaptic input from the off bipolars to the off-ganglion cells in the dark.

The inferences above further suggest that the on-center ganglion cells would be inappropriate for signaling threshold responses, but that the off-ganglion cells could signal small changes in presynaptic activity at threshold. Our threshold measurements at the ganglion cells tend to support this notion: off-ganglion cells are more sensitive than the on-ganglion cells at threshold levels.²

SOURCE OF THE TRANSIENT IPSPs About 80% of the ganglion cells studied in this report responded with transient IPSPs, having a reversal potential near -50 mV, at the onset or termination of center or surround illumination. The IPSP was most apparent in the on-off ganglion cell at all four phases of the response, but the IPSP could also be measured in the other ganglion cell types, although smaller in magnitude and not apparent generally at all four phases. The transient time-course of the IPSP and its broad receptive field properties suggest that it originates in the broad field transient amacrine cells shown in Fig. 3, and described elsewhere in mudpuppy (Werblin, 1977; Werblin and Copenhagen, 1974; Thibos and Werblin, 1978).

The transient hyperpolarizations have been reported previously in ganglion cells, but only in the on-off types (Werblin, 1977). This is probably because the reversal potential for the IPSPs is normally near the dark level, so the driving force is low, and the response is usually small. These PSPs are augmented by membrane polarization and can appear in all ganglion cell types, as shown in these studies.

Miller and Dacheux (1976b) showed that the IPSPs in mudpuppy on-off ganglion cells were reversed by intracellular injection of chloride. Their work together with ours suggests that the IPSPs are mediated by an increase in conductance to chloride, and that the equilibrium potential for chloride is near the ambient potential level. These observations are consistent with other studies of chloride-mediated PSPs where the reversal potential for the response is near the ambient potential level (Coombs et al., 1955).

SOURCE OF THE NARROW FIELD TRANSIENT EXCITATION IS UNCERTAIN The narrow field transient excitatory PSP, measured as a component of the center

² Skrzypet, J. S., and F. S. Werblin. Manuscript in preparation.

response in the on-off and hybrid ganglion cells, is the only component of unknown origin. All transient amacrine cells recorded in tiger salamander have much broader receptive fields. The sustained amacrine cells have quite different response properties,¹ and the bipolars, which characteristically show narrow receptive fields, generate sustained responses.

Therefore, the narrow field transient response is probably formed through the presynaptic interaction of bipolar and/or amacrine cells. This input might represent the interaction of bipolar inputs of opposing phase, as suggested by Toyoda et al. (1973) and Miller and Dacheux (1976c). Alternatively, the transient EPSP might be due to interaction of opposing influences of amacrine cells with slightly different latencies; the excitatory influence leading the inhibitory component by about 100 ms (Werblin, 1977). Other combinations of presynaptic influences are also possible.

This study shows that most forms of ganglion cell activity can be explained in terms of excitatory inputs from bipolars and inhibitory inputs from the transient amacrine cell system. The source of the narrow-field transient excitation, the role of the sustained amacrines, and the identity of the sustained depolarizing surround response in hybrid cells remain to be resolved.

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